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Survival of *Acinetobacter baumannii* on Dry Surfaces

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***Acinetobacter* spp. have frequently been reported to be the causative agents of hospital outbreaks. The circumstances of some outbreaks demonstrated the long survival of *Acinetobacter* in a dry, inanimate environment. In laboratory experiments, we compared the abilities of five *Acinetobacter baumannii* strains, three *Acinetobacter* sp. strains from the American Type Culture Collection (ATCC), one *Escherichia coli* ATCC strain, and one *Enterococcus faecium* ATCC strain to survive under dry conditions. Bacterial solutions of the 10 strains were inoculated onto four different material samples (ceramic, polyvinyl chloride, rubber, and stainless steel) and stored under defined conditions. We investigated the bacterial counts of the material samples immediately after inoculation, after drying, and after 4 h, 1 day, and 1, 2, 4, 8, and 16 weeks of storage. A statistical model was used to distribute the 40 resulting curves among four types of survival curves. The type of survival curve was significantly associated with the bacterial strain but not with the material. The ability of the *A. baumannii* strains to survive under dry conditions varied greatly and correlated well with the source of the strain. Strains isolated from dry sources survived better than those isolated from wet sources. An outbreak strain that had caused hospital-acquired respiratory tract infections survived better than the strains from wet sources, but not as well as strains from dry sources. Resistance to dry conditions may promote the transmissibility of a strain, but it is not sufficient to make a strain an epidemic one. However, in the case of an outbreak, sources of *Acinetobacter* must be expected in the dry environment.**

The importance of the gram-negative rod *Acinetobacter* as the cause of nosocomial infections has increased during the last few years (3). In particular, outbreaks due to *Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*) have frequently been reported (1, 2, 6, 7, 15, 18, 20, 21, 23, 25–29, 31, 32). Many of these outbreaks could be traced to environmental sources, e.g., reusable pressure transducers (2), room humidifiers (28), mattresses (27), pillows (32), and frequently, components of ventilation equipment (6, 15, 26, 29, 31). While investigating these outbreaks, it was recognized that at least some *A. baumannii* strains are able to survive for long periods under dry conditions (1, 5). Buxton and coworkers (5) reported on the first indications that *Acinetobacter* spp. can survive over long periods under dry conditions. They identified a ventilation therapist's contaminated washcloth as the probable source of an outbreak. *A. baumannii* was recovered from the washcloth after 7 days of storage under dry conditions (5). Other investigators demonstrated that *A. baumannii* can survive for 6 days on dry filter paper (1), 13 days on formica (12, 19), more than 7 days on glass (16), and more than 25 days on cotton (16). However, those investigations did not clarify whether the ability to survive is a characteristic of the species or differs from strain to strain, making some strains more likely to become responsible for epidemics. Therefore, we studied the ability of different *Acinetobacter* strains to survive under dry conditions and compared them with other gram-negative and gram-positive species. Since surfaces may influence the ability to survive (9, 11, 16), all experiments were performed on surfaces typical of those in the hospital environment.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. Five genetically distinct *A. baumannii* wild strains (demonstrated by a pulsed-field gel electrophoresis method which has been described elsewhere [22]) isolated from patients and the environment and five strains from the American Type Culture Collection (ATCC) were used for the experiments (Table 1). The wild strains were selected from a collection of nearly 200 *Acinetobacter* isolates collected over a period of 2 years. We selected two strains from wet sources (urine and sewage), two isolates from dry surfaces (working surface and pillowcase), and one epidemic strain. The epidemic strain was the causative agent of an outbreak of respiratory tract colonization or infection that lasted nearly 3 years. This outbreak could be traced to contaminated temperature probes from mechanical ventilation equipment.

We used an *A. baumannii* strain, two strains of other *Acinetobacter* spp. (*Acinetobacter junii* and *Acinetobacter* genospecies 3), one *Escherichia coli* strain, and one *Enterococcus faecium* strain, all of which were from ATCC.

All strains were stored at −70°C in glycerol broth. All strains except the *E. faecium* strain were grown on trypton soy plates at 37 ± 2°C for 24 h; the *E. faecium* strain was grown on kanamycin esculin azide plates.

The susceptibilities of the strains to ampicillin, ampicillin-sulbactam, piperacillin, mezlocillin, cefotiam, cefotaxime, ceftriaxon, cefaclor, ceftacidime, imipenem, meropenem, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, ofloxacin, ciprofloxacin, tetracycline, amikacin, and fosfomycin were tested. In addition, the susceptibility of *E. faecium* to erythromycin, vancomycin, tobramycin, and clindamycin was tested. All tests were performed by the bouillon microdilution method.

Preparation of bacterial inocula. The bacteria were cultured overnight, washed, and suspended in sterile distilled water. Distilled water was used to avoid protective factors that may be components of other solutions (e.g., proteins in broth). The bacterial concentration was adjusted to 10^8 CFU/ml by photometric measurement of solution turbidity. The final concentrations were confirmed by a dilution series and averaged 8×10^7 CFU/ml.

Surface inoculation and culturing. Ceramic, polyvinyl chloride (PVC), rubber, and stainless steel samples (5 by 5 cm) were disinfected with 70% ethanol and contaminated with 0.1 ml of the bacterial solution. The total number of contaminated samples was 1,800 (45 samples per strain and material). All samples were stored in a dark, dust-protected climate chamber at 22 ± 2°C with 50% ± 5% relative humidity. At various time intervals—immediately after drying (zero time), after 4 hours, after 1 day, and after 1, 2, 4, 8, and 16 weeks—viable cells were recovered from the contaminated samples. For that purpose, from a total of 45 contaminated samples per strain and material, 5 samples were randomly chosen and shaken for 5 min in 100 ml of a 0.9% sodium chloride solution containing sterile glass beads. The bacterial concentration in the solution was determined in duplicate by membrane filtration and/or a dilution series. The number of bacteria per sample was calculated from the results.

To evaluate the performance of this method, five samples per strain and

TABLE 1. Description of the strains used in the study and their sources

Strain	No. of isolates	Source	Drugs to which isolate was resistant	Comments
A. baumannii	58	Tracheal secretion	Gentamicin, cefotiam, cefaclor, fosfomycin	Strain was epidemic on an intensive care unit over 2.5 yr
A. baumannii	156	Urine	Ampicillin, trimethoprim-sulfamethoxazole, tobramycin, mezlocillin, cefotiam, cefotaxime, ceftriaxon, cefaclor, tetracycline, ofloxacin, ciprofloxacin	Sporadic isolate
A. baumannii	166	Pillow	Cefotiam	
A. baumannii	167	Working surface	Ampicillin, trimethoprim-sulfamethoxazole, gentamicin, mezlocillin, cefotiam, cefotaxime, cefaclor	
A. baumannii	7B	Sewage		
A. baumannii	ATCC 19606	Urine	Ampicillin, trimethoprim-sulfamethoxazole, gentamicin, mezlocillin, cefotiam, cefotaxime, cefaclor	
A. junii	ATCC 17908	Urine	Cefotiam	
A. genospecies 3	ATCC 17822	Unknown	Ampicillin, mezlocillin, cefotiam, cefaclor	
E. coli	ATCC 11775	Urine		
E. faecium	ATCC 19433	Unknown	Gentamicin, tobramycin, cefotiam, cefotaxime, ceftriaxon, cefaclor, ceftacidime, amikacin, imipenem, ofloxacin	

material were investigated immediately after contamination to assess the recoverable proportion of bacteria.

Statistical analysis. The results were calculated as the average of the bacterial counts per culture time, strain, and material. The recoverable proportion was calculated as a percentage of the inoculated colony counts. The reduction after desiccation was calculated as a reduction factor by using the formula $\log_{10} K_o - \log_{10} K_a$, where K_o equals the recoverable colony count and K_a is the colony count after drying. The influence of material and bacterial strain on the recoverable proportion and the reduction after desiccation was investigated by using a two-factorial random-effect analysis of variances (ANOVA) model (SPSS for Windows; SPSS Inc., Chicago, Ill.).

Using the colony counts from the different culturing intervals (zero time to 16 weeks), we prepared 40 survival curves (10 bacterial strains on four materials). These survival curves were analyzed by a finite-mixture model and were classified into different types (10). The correlation between type of survival curve and material and type of survival curve and strain was analyzed by the chi-square test. All tests were performed two-tailed, and α was set at 0.05.

RESULTS

Recoverable proportion. The recoverable proportion of the strains on the different materials varied greatly, ranging from 12% (*A. baumannii* ATCC 19606 on ceramic) to 96% (*A. baumannii* 7B on rubber). On the average, 46% of the inoculated microorganisms were recovered. The random-effect ANOVA model showed significant interaction between material and strain. Because an interaction was detected, the effects of the material or the strains were not investigated.

Reduction by desiccation. Desiccation reduced the colony counts by 0.3 $\log_{10}$ steps (*A. baumannii* 167 on PVC) to 6.9 $\log_{10}$ steps (*A. baumannii* 7B on rubber). The *A. baumannii* 7B strain isolated from sewage was the most susceptible strain on all materials.

The mean reduction for all strains on all materials was 4 $\log_{10}$ steps. The random-effect ANOVA model revealed an interaction between strain and material. Therefore, we did not analyze the influence of strains or material.

Types of survival curves. Forty survival curves (10 strains on four materials) were characterized by the fact that 50% of them showed survival at relevant colony counts of more than 10^2 CFU per sample for at least 2 weeks. Using a finite-mixture model, we separated the survival curves into four types (Fig. 1). Type 1 (five curves) was distinguished by long survival times at high colony counts. It showed about 10^7 CFU per material sample after 4 months of storage. Type 2 (five curves) also showed long survival times, but mean colony counts were lower than those for type 1 and a higher level of reduction was observed. Type 3 (12 curves) started with colony counts of about 10^5 CFU per material sample and was close to zero at the end of the observation period. Although survival curve type 4 (18 curves) also had colony counts near zero at the end of the observation period, the curves were more shallow and started from lower colony counts of about 10^4 CFU per material sample.

In contrast to the influence of materials on the recoverable proportion and the reduction by drying, no such influence was noted in association with the types of survival curves ($P = 0.41$; chi-square test). Instead, the types of survival curves were homogeneously distributed among materials.

However, we found a significant association between types of survival curves and bacterial strains ($P < 0.0005$; chi-square test) (Table 2). Strains isolated from sewage and urine (*A. baumannii* 7B and *A. baumannii* 156, respectively) were susceptible to dry conditions and comprised survival curves of type 3 and 4. *A. baumannii* 166 from a pillow and *A. baumannii* 167 from a working surface were resistant to dry conditions. These strains comprised type 1 or 2, but *A. baumannii* 166 on stainless steal comprised survival curves of type 4. *A. baumannii* 58, which had caused the long persistent outbreak, had a moderate resistance to dry conditions. It comprised survival curves of type 2 and 3. All ATCC strains except *E. faecium* were susceptible to dry conditions and comprised curves of type 3 or 4; *E. faecium* comprised survival curve type 2 once.

DISCUSSION

The taxonomy of the genus *Acinetobacter* was indeterminate until the early 1970s. The epidemiology of these organisms was initially investigated by implementing a standardized taxonomy. Although the interest in *Acinetobacter* spp. as a nosocomial pathogen has increased, little is known about its natural reservoirs and mode of transmission.

However, in some investigations, it was found that some *Acinetobacter* strains may survive on a hospital unit for as long as several years (17, 27, 32). One reason for this persistence may be the resistance of *Acinetobacter* to many antibiotics (4).

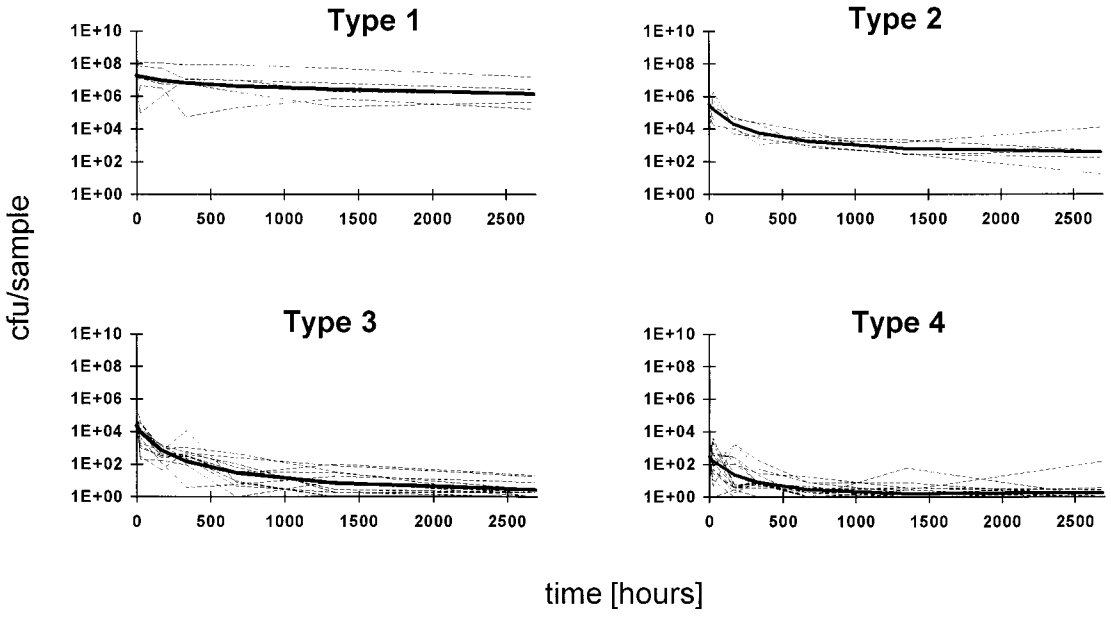

FIG. 1. Distribution of 40 survival curves (dotted lines) among four types of survival curves by using a finite-mixture model. Solid bold lines, statistically derived types of survival curves; *x* axis, survival time in hours; *y* axis, numbers of CFU/sample.

In some outbreaks, drastic measures (e.g., closing the ward [30] and extensive disinfection of the environment [8, 13, 24]) were needed to terminate the outbreak. Such refractory persistence of *Acinetobacter* may be due to unrecognized reservoirs, e.g., in the dry inanimate environment.

Recently, it has been suggested for another gram-negative nosocomial pathogen (*Burkholderia cepacia*) that epidemic strains survive longer than sporadic strains on dry surfaces, leading to better transmissibility and thus to the appearance of epidemics (11).

In our experiments, we compared the abilities of an epidemic *A. baumannii* strain and sporadic strains to survive. To take into account the fact that different surfaces may influence the survival times, we used materials that are typical for the hospital environment.

It was found that the surfaces only influenced the recoverable proportion of microorganisms and the reduction by desiccation. The extent to which the material surface permits adhesion may be responsible for the differences in the recoverable proportion. The influence of the material on the reduction by drying may be due to substances (e.g., softening agents from PVC or rubber or the iron ions from steel) that dissolve in the bacterial solution and that are toxic for the microorganisms (11, 14). However, this phenomenon has not yet been fully elucidated and requires further investigation.

The long-term survival of the investigated microorganisms under dry conditions was not influenced by the material. This is in contrast to the results of Drabick and coworkers (11), who found that the material significantly influenced the ability of *B. cepacia* to survive under dry conditions. Their observation time was no longer than 24 h and thus may have been too short to separate the effects caused by drying from those that influenced the ability for long-term survival under dry conditions.

The ability of different strains of *A. baumannii* to survive under dry conditions appears to vary greatly. Some strains were able to survive for several months without any reduction in colony counts, whereas other strains hardly survived the process of drying. This feature of the strain correlated well with the source from which the strain was isolated. Strains isolated from wet sources did not survive as well as those isolated from dry sources. The reason for the variability in the ability to survive under dry conditions among the strains of a single species remains unclear and requires further study.

Acinetobacter ATCC strains did not survive very well under dry conditions compared with most of the other *Acinetobacter* strains. This may be due to the fact that the ATCC strains were isolated from a wet source (urine). In a similar manner, the ability of *E. coli* isolated from urine to survive corresponded to that of *A. baumannii* strains isolated from wet sources. Surprisingly, the studied *E. faecium* strain, which is usually resistant to environmental influences, did not survive very well under dry conditions. However, as our results from studies with *Acinetobacter* spp. suggest, it is not possible to unreservedly draw conclusions about the entire species from the results obtained with one strain.

The epidemic *A. baumannii* strain survived under dry conditions better than the susceptible strains from wet sources, but not as well as the resistant strains from dry sources. Resistance

TABLE 2. Association between strain and type of survival curve

Strain	No. of curves of the following type:			
	Type 1	Type 2	Type 3	Type 4
A. baumannii 58		2	2	
A. baumannii 156			3	1
A. baumannii 166	2	1		1
A. baumannii 167	3	1		
A. baumannii 7B				4
A. baumannii ATCC 19606			4	
A. junii ATCC 17908				4
Acinetobacter genospecies 3 ATCC 17822			1	3
E. coli ATCC 11775			2	2
E. faecium ATCC 19433		1		3

to dry conditions may promote the occurrence of outbreaks, but this feature alone does not seem sufficient to make a strain an epidemic strain.

Our experiments did not simulate the hospital environment exactly. The inoculated bacterial concentration was relatively high and probably exceeded the amount of contaminating bacteria that may occur in the hospital. On the other hand, the inoculated microorganisms were depleted of protective factors, e.g., respiratory secretions (11).

In conclusion, our experiments demonstrated that some *Acinetobacter* strains may survive for more than 4 months under dry conditions. The ability to survive under dry conditions seems to be a characteristic of the strain rather than of the species and was not influenced by the surfaces. Thus, in the case of prolonged outbreaks, the sources of *Acinetobacter* strains should be expected to be the patients' inanimate dry environment, so that extensive disinfection may be required to confine the outbreaks.

REFERENCES

1. **Allen, K. D., and H. T. Green.** 1987. Hospital outbreak of multi-resistant Acinetobacter anitratus: an airborne mode of spread? J. Hosp. Infect. **9:**110–119.
2. **Beck-Sague, C. M., W. R. Jarvis, J. H. Brook, D. H. Culver, A. Potts, E. Gay, B. W. Shotts, B. Hill, R. L. Anderson, and M. P. Weinstein.** 1990. Epidemic bacteremia due to Acinetobacter baumannii in five intensive care units. Am. J. Epidemiol. **132:**723–733.
3. **Bergogne-Berezin, E., and M. L. Joly-Guillou.** 1991. Hospital infection with Acinetobacter spp.: an increasing problem. J. Hosp. Infect. **18**(Suppl. A)**:** 250–255.
4. **Buisson, Y., G. Tran Van Nhieu, L. Ginot, P. Bouvet, H. Schill, L. Driot, and M. Meyran.** 1990. Nosocomial outbreaks due to amikacin-resistant tobramycin-sensitive Acinetobacter species: correlation with amikacin usage. J. Hosp. Infect. **15:**83–93.
5. **Buxton, A. E., R. L. Anderson, D. Werdegar, and E. Atlas.** 1978. Nosocomial respiratory tract infection and colonization with Acinetobacter calcoaceticus—epidemiologic characteristics. Am. J. Med. **65:**507–513.
6. **Cefai, C., J. Richards, F. K. Gould, and P. McPeake.** 1990. An outbreak of Acinetobacter respiratory tract infection resulting from incomplete disinfection of ventilatory equipment. J. Hosp. Infect. **15:**177–182.
7. **Contant, J., E. Kemeny, C. Oxley, E. Perry, and G. Garber.** 1990. Investigation of an outbreak of Acinetobacter calcoaceticus var. anitratus infections in an adult intensive care unit. Am. J. Infect. Control **18:**288–291.
8. **Crombach, W. H., L. Dijkshoorn, M. van Noort-Klaassen, J. Niessen, and G. van Knippenberg-Gordebeke.** 1989. Control of an epidemic spread of a multi-resistant strain of Acinetobacter calcoaceticus in a hospital. Intensive Care Med. **15:**166–170.
9. **de Veer, I., K. Wilke, and H. Rüden.** 1993. Bactericide qualities of materials containing and not containing copper I. Communication: examinations about contamination and sedimentation under wet and dry conditions. Zentralb. Hyg. Umweltmed. **195:**66–87.
10. **Dietz, E., and D. Böhning.** 1994. Analysis of longitudinal data using a finite mixture model. Stat. Papers **35:**203–210.
11. **Drabick, J. A., E. J. Gracely, G. J. Heidecker, and J. J. LiPuma.** 1996. Survival of Burkholderia cepacia on environmental surfaces. J. Hosp. Infect. **32:**267–276.
12. **Getchell-White, S. I., L. G. Donowitz, and D. M. H. Gröschel.** 1989. The inanimate environment of an intensive care unit as a potential source of nosocomial bacteremia: evidence for long survival of Acinetobacter calcoaceticus. Infect. Control Hosp. Epidemiol. **10:**402–407.
13. **Go, E. S., C. Urban, J. Burns, B. Kreiswirth, W. Eisner, N. Mariano, K. Mosinka-Snipas, and J. J. Rahal.** 1994. Clinical and molecular epidemiology of Acinetobacter infections sensitive only to polymyxin B and sulbactam. Lancet **344:**1329–1332.
14. **Gundermann, K. O., and H. Johannsen.** 1970. Untersuchungen zur Überlebensdauer von Bakterien auf Oberflächen und der Möglichkeiten ihrer Beeinflussung. Arch. Hyg. **2:**102–109.
15. **Hartstein, A. I., A. L. Rashad, J. M. Liebler, L. A. Actis, J. Freeman, J. W. Rourke, Jr., T. B. Stibolt, M. E. Tolmasky, G. R. Ellis, and J. H. Crosa.** 1988. Multiple intensive care unit outbreak of Acinetobacter calcoaceticus subspecies anitratus respiratory infection and colonization associated with contaminated, reusable ventilator circuits and resuscitation bags. Am. J. Med. **85:** 624–631.
16. **Hirai, Y.** 1991. Survival of bacteria under dry conditions; from a viewpoint of nosocomial infection. J. Hosp. Infect. **19:**191–200.
17. **Holton, J.** 1982. A report of a further hospital outbreak caused by a multiresistant Acinetobacter anitratus. J. Hosp. Infect. **3:**305–309.
18. **Kropec, A., J. Hubner, and F. D. Daschner.** 1993. Comparison of three typing methods in hospital outbreaks of Acinetobacter calcoaceticus infection. J. Hosp. Infect. **23:**133–141.
19. **Musa, E. K., N. Desai, and M. W. Casewell.** 1990. The survival of Acinetobacter calcoaceticus inoculated on fingertips and on formica. J. Hosp. Infect. **15:**219–227.
20. **Ng, P. C., R. A. Herrington, C. A. Beane, A. T. Ghoneim, and P. R. Dear.** 1989. An outbreak of acinetobacter septicemia in a neonatal intensive care unit. J. Hosp. Infect. **14:**363–368.
21. **Patterson, J. E., J. Vecchio, E. L. Pantelick, P. Farrel, D. Mazon, M. J. Zervos, and J. W. J. Hierholzer.** 1991. Association of contaminated gloves with transmission of Acinetobacter calcoaceticus var. anitratus in an intensive care unit. Am. J. Med. **91:**479–483.
22. **Pfaller, M., R. J. Hollis, and H. S. Sader.** 1992. PFGE of chromosomal DNA, section 10.15.c. *In* H. D. Isenberg (ed.), Clinical microbiological procedures handbook. American Society for Microbiology, Washington, D.C.
23. **Sakata, H., K. Fujita, S. Maruyama, H. Kakehashi, Y. Mori, and H. Yoshioka.** 1989. Acinetobacter calcoaceticus biovar anitratus septicemia in a neonatal intensive care unit: epidemiology and control. J. Hosp. Infect. **14:**15–22.
24. **Scerpella, E. G., A. R. Wanger, L. Armitige, P. Anderlini, and C. D. Ericsson.** 1995. Nosocomial outbreak caused by a multiresistant clone of Acinetobacter baumannii: results of the case-control and molecular epidemiologic investigations. Infect. Control Hosp. Epidemiol. **16:**92–97.
25. **Schloesser, R. L., E. A. Laufkoetter, T. Lehners, and C. Mietens.** 1990. An outbreak of Acinetobacter calcoaceticus infection in a neonatal care unit. Infection **18:**230–233.
26. **Shelly, M. P., G. R. Park, R. E. Warren, and R. J. Whetstone.** 1986. Portable lung ventilators: the potential risk from bacterial colonization. Crit. Care Med. **12:**328–331.
27. **Sherertz, R. J., and M. L. Sullivan.** 1985. An outbreak of infections with Acinetobacter calcoaceticus in burn patients: contamination of patients' mattresses. J. Infect. Dis. **151:**252–258.
28. **Smith, W., and R. M. Massanari.** 1977. Room humidifiers as the source of Acinetobacter infections. JAMA **237:**795–797.
29. **Stone, J. W., and B. C. Das.** 1985. Investigation of an outbreak of infection with Acinetobacter calcoaceticus in a special care baby unit. J. Hosp. Infect. **6:**42–48.
30. **Tankovic, J., P. Legrand, G. De Gatines, V. Chemineau, C. Brun-Buisson, and J. Duval.** 1995. Characterization of a hospital outbreak of imipenem-resistant *Acinetobacter baumannii* by phenotypic and genotypic typing methods. J. Clin. Microbiol. **32:**2677–2681.
31. **Vandenbroucke-Grauls, C. M., A. J. Kerver, J. H. Rommes, R. Jansen, C. den Dekker, and J. Verhoef.** 1988. Endemic Acinetobacter anitratus in a surgical intensive care unit: mechanical ventilators as reservoir. Eur. J. Clin. Microbiol. Infect. Dis. **7:**485–489.
32. **Weernink, A., W. P. J. Severin, I. Tjernberg, and L. Dijkshoorn.** 1995. Pillows, an unexpected source of Acinetobacter. J. Hosp. Infect. **29:**189–199.